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Holtmaat et al., (1996) Brain. Res. Mol. Brain Res. 41, 148-156).

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Functional Expression of Soluble Human Interleukin-11 (IL-11) Receptor α and Stoichiometry of *in Vitro* IL-11 Receptor Complexes with gp130*

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The interleukin-6 (IL-6) family of cytokines activates signaling through the formation of either gp130 homodimers, as for IL-6, or gp130-leukemia inhibitory factor receptor (LIFR) heterodimers as for ciliary neurotrophic factor (CNTF), leukemia inhibitory factor, oncostatin^M, and cardiotrophin-1. Recent *in vitro* studies with IL-6 and CNTF have demonstrated that higher order hexameric receptor complexes are assembled in which signaling chain dimerization is accompanied by the dimerization of both the cytokine molecule and its specific receptor α subunits (IL-6Rα or CNTFRα, respectively). IL-11 is a member of the IL-6 family and known to require gp130 but not LIFR for signaling. In this study we investigate the functional and biochemical composition of the IL-11 receptor complex. The human IL-11 receptor α-chain was cloned from a human bone marrow cDNA library. IL-11Rα was shown to confer IL-11 responsiveness to human hepatoma cells either by cDNA transfection or by adding a soluble form of the receptor (sIL11Rα) expressed in the baculovirus system to the culture medium. *In vitro* immunoprecipitation experiments showed that sIL11Rα specifically binds IL-11 and that binding is enhanced by gp130. Similarly to IL-6 and CNTF, gp130 is able to induce dimerization of the IL-11·IL-11Rα subcomplex, the result of which is the formation of a pentameric receptor complex. However, in contrast to the other two cytokines, IL-11 was unable to induce either gp130 homodimerization or gp130/LIFR heterodimerization. These results strongly suggest that an as yet unidentified receptor β-chain is involved in IL-11 signaling.

Interleukin (IL)-11[1] was originally identified and molecularly cloned on the basis of its ability to stimulate proliferation of the IL-6-dependent mouse plasmacytoma cell line, T1165 (1). Some of the biological activities of IL-11 (for review see Ref. 2) are also elicited by IL-6 (3) and, to a lesser extent, by leukemia inhibitory factor (LIF), oncostatin M (OSM), and ciliary neurotrophic factor (CNTF) (4). This biological pleiotropy and redundancy, characteristic of many cytokines, suggest that components of signal transduction pathways or receptor complexes must be shared by these growth factors. Previous studies have indicated that IL-11 depends on gp130 to transduce its signals into cells (5). gp130 is an IL-6 receptor-associated signal transducer, which is also a component of the high affinity receptor complexes of LIF, CNTF, OSM, and cardiotrophin-1 (6–9). The receptors of this family of cytokines are activated by either homodimerization of gp130, as in the case of IL-6 (10), or by heterodimerization between gp130 and a second gp130-related protein known as LIFR, as in the case of LIF, OSM, and CNTF (11, 12). Such homo- or heterodimers activate the Jak-Tyk family of cytoplasmic tyrosine kinases, which are constitutively associated with gp130 or LIFR (13, 14) and induce overlapping but distinct patterns of tyrosine phosphorylation (7, 15, 16). APRF (acute phase response factor), also called STAT3 (17), is preferentially phosphorylated in response to the IL-6 cytokine family (18, 19) and belongs to the signal transducers and activators of transcription (STAT) family, which links the signaling pathway of activated receptor complexes to the nuclear apparatus for transcriptional regulation (20).

Cell surface receptor assembly is often a sequential multistep process. Each cytokine first interacts with a ligand-binding subunit, followed by association with signal-transducing β-components. Ligand-specific receptor α-chains have been identified for IL-6 (21) and CNTF (22). Interestingly, both these receptor subunits, while acting as docking molecules for their respective cytokines, do not directly contribute to intracellular signaling (23, 24). In particular, CNTFRα is expressed as a glycosylphosphatidylinositol membrane-linked receptor that lacks transmembrane and intracytoplasmic domains (22). As a consequence, soluble extracytoplasmic forms of IL-6Rα and CNTFRα potentiate cytokine activity because they allow signaling-competent β-chain dimers to be formed also in cells that do not naturally express membrane-bound α-chains (23, 25). Recently, a murine IL-11 receptor α-chain was also cloned (26, 27), followed by the molecular identification of its human counterpart (28, 29). IL-11Rα shares substantial sequence and structural similarities with IL-6Rα and CNTFRα, suggesting functional equivalence of this receptor chain in IL-11 biological activity and signaling activation.

The stoichiometry of the high affinity receptor complexes is unknown for most cytokines. Recently, however, biochemical studies (30) as well as solution phase binding assays (31) have identified the IL-6 signal-transducing receptor as a hexameric complex, consisting of two IL-6 molecules, two IL-6 receptor α-chains, and a homodimer of gp130. Using the same approach, a similar hexamer of two CNTF molecules, two CNTF receptor α-chains, and a heterodimer of gp130 and LIFR was proposed for CNTF signaling (32). The question thus arises of whether

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[1] The abbreviations used are: IL, interleukin; IL-11R, IL-11 receptor; sIL-11R, soluble IL-11 receptor; LIF, leukemia inhibitory factor; OSM, oncostatin M; CNTF, ciliary neurotrophic factor; APRF, acute phase response factor; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; bp, base pair(s); STAT, signal transducers and activators of transcription; R, receptor.

also IL-11, which requires both IL-11Rα and gp130, assembles *in vitro* hexameric receptor complexes.

The aim of this work was to study the biological properties of IL-11Rα and its role in IL-11 receptor assembly *in vitro*. The coding region of the human IL-11 receptor gene was isolated from a bone marrow cDNA library, and a soluble form of the IL-11 receptor (sIL11Rα) was expressed in the baculovirus system. Human sIL11Rα was able to confer IL-11 responsiveness to hepatoma cells in culture and specifically interacted *in vitro* with IL-11 and gp130. Analysis of the stoichiometry of the individual components of the complex revealed that the IL-11·sIL11Rα subcomplex undergoes dimerization in the presence of gp130, thereby assembling a pentameric receptor complex, but that it was not possible to induce either gp130 homodimerization or gp130/LIFR heterodimerization *in vitro*.

EXPERIMENTAL PROCEDURES

Cytokines—Human recombinant granulocyte macrophage colony stimulating factor and recombinant human interleukin-11 (IL-11) were purchased from Pepro Tech Inc. Recombinant human ciliary neurotrophic factor (CNTF) was generously provided by R. Laufer. Interleukin-6 (IL-6) was prepared as described (33).

Tissue Culture—The human erythroleukemic cell line TF1 was cultured in RPMI 1640 medium containing 10% fetal calf serum supplemented with 1 ng/ml human recombinant granulocyte-macrophage colony-stimulating factor. Hep3B cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Both cell lines were cultured at 37 °C in the presence of 5% CO_2.

Preparation of cDNA from Total RNA—Total RNA was isolated from 4×10^7 TF1 cells or from mouse kidney using the ULTRASPEC-II RNA Isolation System (Biotecx Laboratories), according to the instructions of the manufacturer. Reverse transcription of RNA was carried out with 1000 units of Moloney murine leukemia virus reverse transcriptase, 5 μg of total TF1 RNA or mouse kidney RNA, 100 pmol of oligo$(dT)_{12}$MN (M: G, A, C; and N: G, A, T, C) in 20 μl of 10 mM dithiothreitol, 50 mM Tris/HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, and 0.2 mM of each dNTPs. The mixture was incubated for 1 h at 37 °C, and the enzyme was inactivated by heating for 5 min at 80 °C.

Cloning of the Human IL-11 Receptor—A pair of fully degenerate PCR primers (P4s, 5′-GCGGATCCTA(T/C)GTNTG(C/T)CA(A/G)ACN-3′; P7a, 5′-GCTCTAGAGG(G/A)CANGGCCANGG-3′) derived from the amino acid sequence of the murine IL-11 receptor (26) was used for PCR on TF1 cDNA prepared as described above. A 255-bp PCR fragment showed high homology with the published mouse IL-11 receptor sequence. This DNA fragment and a 221-bp murine PCR fragment (nucleotides 1144–1364) were used as DNA probes to screen about 5×10^5 plaques of a human bone marrow cDNA library (Human Bone Marrow 5′-Stretch cDNA library, Clontech). Several positive plaques were isolated, and the inserts of three independent positive plaques (clones 5′-1, 8e, and rec3/4) were subcloned into the *Bam*HI/*Xba*I sites of pGEM-3Zf+ (Promega) and sequenced on both strands using internal primers. Further details on the structure of these clones are given in Fig. 1.

Reconstruction of the two clones 5′-8e and 5′-rec of IL11Rα cDNA was achieved by joining clone 5′-1 with clone 8e or rec3/4, respectively.

Expression Constructs—The 1277- and 1530-bp cDNA fragments of clones 5′-8e and 5′-rec were inserted into the eukaryotic expression vector pHD (34) for transient transfection experiments. The resultant constructs were named pHD-8e and pHD-rec, respectively.

For the expression of tagged forms of the soluble IL-11 receptor in insect cells, myc (35) or FLAG epitopes (36) were added to the C terminus of the protein by PCR. The resulting C termini were, in the case of myc, E(364)-FEEQKLISEEDL-Stop (hereafter called sIL11R-myc) and, in the case of FLAG, E(323)-FDYKDDDDK-Stop (hereafter called sIL11R-flag). The cDNAs of the soluble receptor variants sIL11R-myc and sIL11R-flag were then cloned into the baculovirus expression vector pBlueBacIII (Invitrogen).

For *in vitro* transcription/translation, a cDNA insert coding for human IL-11 obtained by PCR was cloned in-frame into the expression vector pT7.7 (clone pT7.7-IL11) (37). The resulting recombinant protein started with the first methionine from pT7.7 followed by the proline at position 22 of the original IL-11 sequence (Swiss-Prot: P20809). Human recombinant IL-11 was expressed in bacteria using the expression vector pFLAG-MAC (Biosystems), into which the coding sequence for mature human IL-11 was inserted (clone pFLAG-IL11).

DNA Transfection Experiments—DNA transfections of Hep3B cells were performed with the calcium phosphate precipitation technique (38). 1×10^6 Hep3B cells were transfected with 20 μg of pHD, pHD-8e, or pHD-rec and 6 μg of the reporter gene 4x$T\alpha_2$M-Luc (39). After 16 h, cells from a single transfection were divided into aliquots for control and cytokine treatments. After 20 h of induction, cells were harvested, and extracts were prepared in 250 mM Tris/HCl, pH 7.8, by repeated freezing and thawing. Total protein concentration was determined using the Bradford colorimetric assay (Bio-Rad). Luciferase assay was performed with 20 μg of protein as described (40).

Expression of Recombinant Soluble IL-11 Receptor—The generation of recombinant baculovirus expressing the two tagged soluble IL-11 receptor variants was performed as described (31).

Gel Retardation Assays—Double-stranded oligonucleotides M67 (41) were labeled by filling in 5′-protruding ends with T7-DNA polymerase, using [α-^{32}P]dATP and [α-^{32}P]dCTP (both 3000 Ci/mmol). Whole cell extract was prepared as follows. Cells were washed once with ice-cold phosphate-buffered saline (PBS) containing 0.1 M sodium fluoride (NaF) and detached from the plates with ice-cold TEN (40 mM Tris, pH 7.5, 1 mM EDTA, 150 mM NaCl) containing 0.1 M NaF. After centrifugation, the cell pellet was frozen in liquid nitrogen. Whole cell extract from about 1×10^6 cells was prepared using 100 μl of extraction buffer (10 mM Hepes, pH 7.9, 400 mM NaCl, 0.1 mM EGTA, 5% glycerol, 100 mM NaF, 10 mM tetrasodium diphosphate, 0.5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, and 10 μg/ml aprotinin). The extract was centrifuged for 15 min at 50,000 rpm in a TLA 100 rotor (Beckman), and 10 μg of protein was incubated with 3 μg of poly(dI-dC) in gel shift incubation buffer (13 mM Hepes, pH 7.9, 65 mM NaCl, 0.15 mM EDTA) for 10 min on ice. After the preincubation, about 5 fmol (20,000 cpm) of probe was added and the incubation continued for 15 min at room temperature in a final volume of 20 μl. After the incubation, 5 μl of 20% Ficoll were added, and the protein-DNA complexes were separated on a 5% polyacrylamide gel in 0.5 × TBE (1 × TBE is 90 mM Tris base, 90 mM boric acid, 2 mM EDTA). Electrophoresis was performed in 0.5 × TBE at 10 V/cm for 2 h. The gels were then dried and autoradiographed.

Expression of Recombinant Human IL-11—An overnight culture of *E. coli* containing pFLAG-IL11 was diluted 1:100, and when the absorbance at 600 nm reached 0.6, isopropyl thiogalactopyranoside was added to a final concentration of 0.5 mM, and incubation was continued for 4 h. Cells were then collected by centrifugation, resuspended in buffer A (50 mM Tris/HCl, pH 7.5, 1 mM EDTA, 1 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 10% glycerol) plus 20 mM NaCl and broken by two passes through a French press. After centrifugation at 16,000 × *g* for 30 min, the supernatant was loaded onto a heparin-Sepharose column (CL-6B, Pharmacia Biotech Inc.) equilibrated with the same buffer. The column was washed with four volumes of buffer A plus 20 mM NaCl and eluted with a linear gradient of 0.02–0.5 M NaCl in buffer A. Fractions containing the activity were diluted 5-fold with buffer A and loaded onto a 5-ml HiTrap heparin column (Pharmacia), equilibrated with buffer A containing 50 mM NaCl. Protein was eluted in one step with buffer A containing 400 mM NaCl. Active fractions contained about 10 μg/ml IL-11 (tested for biological activity on Hep3B cells transfected with the human IL-11 receptor, data not shown).

^{35}S Labeling of Recombinant Protein—Recombinant soluble receptors were metabolically labeled as described previously (31), except that labeling of sIL11R was performed in methionine-/cysteine-free SF-900-II (Life Technologies, Inc.) medium containing 50 μl of PRO-MIX (specific activity >1000 Ci/mmol, 14.3 mCi/ml) and 50 μl of [^{35}S]cysteine (specific activity >1000 Ci/mmol, 10 mCi/ml) (Amersham Corp.) in a final volume of 2 ml. All other soluble receptors and the cytokine IL-6 were labeled as described previously (31). ^{35}S labeling of IL-11 was obtained by *in vitro* translation with rabbit reticulocyte lysate (Promega).

Immunoprecipitations—Immunoprecipitations were performed as described (31). Briefly, 500 μl of baculovirus supernatant containing the myc-tagged receptor or 100 μl of partially purified recombinant IL-11 containing the FLAG-epitope at the N terminus of the protein were incubated overnight at 4 °C with 50 μl of protein A-Sepharose in phosphate-buffered saline (PBS) (50% v/v) and 4 μl of the anti-myc 9E10 monoclonal antibody (35) or 4 μl of the anti-FLAG monoclonal antibody (Anti-FLAG M2, Kodak), respectively. After washing three times with PBSTB (1 × PBS, 0.05% Tween 20, 0.2% Brij 96), the Sepharose beads were incubated with other receptor(s) and/or cytokine(s) for at least 12 h at 4 °C. The beads were then washed three times with PBSTB, resuspended in SDS loading dye buffer, heated for 5 min at 95 °C, subjected to SDS-polyacrylamide gel electrophoresis, and autoradiographed.

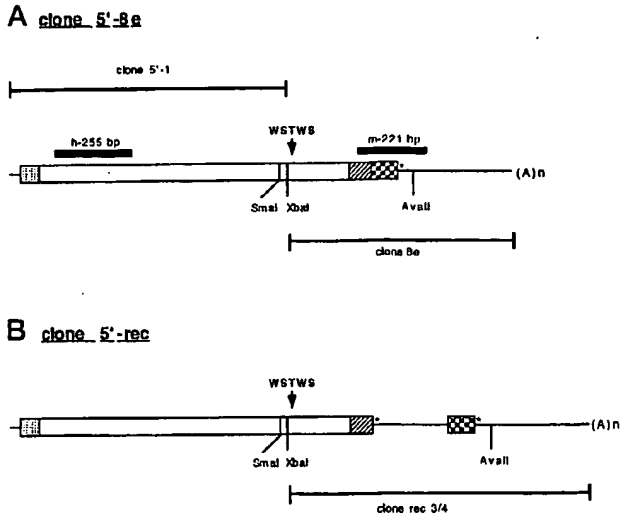

FIG. 1. **Schematic representation of clone 5'-8e and clone 5'-rec.** *Solid lines* show 5'- and 3'-nontranslated and intron region. Coding regions are *boxed*. The signal peptide (▩), the extracellular domain (□), the transmembrane domain (▨), and the cytoplasmic domain (▩) are designated. * indicates translational stop codons. The approximate position of the WSXWS motif conserved among members of the hemopoietin receptor family is shown *above*, and approximate positions of endogenous restriction sites are shown *below*. *A*, structure of the 5'-8e cDNA clone. *Solid bars* designate the position of clone 5'-1 and clone 8e. *Solid boxes* show the approximate position of the human 255-bp and the mouse 221-bp hybridization probe. *B*, structure of clone 5'-rec. *Solid bar* indicates the position of clone rec3/4.

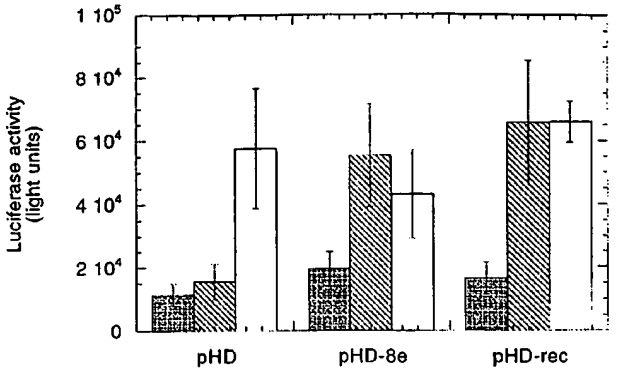

FIG. 2. **Activity of human IL-11 receptor in Hep3B cells.** Hep3B cells were transfected with $4xTa_2M$-Luc and the expression vectors pHD, pHD-8e, or pHD-rec. Subcultures were treated after transfection with medium alone (*control*, ▨), with IL-11 (50 ng/ml, ▩), or IL-6 (10 ng/ml, □) cytokine. The *ordinate* shows absolute luciferase activity in light units per standard assay, normalized for constant amounts of protein (20 μg). Different degrees of induction were due to different transfection efficiencies. Transfections were performed in triplicate and shown is the average of the experiments.

RESULTS

Cloning of the Human IL-11 Receptor—The human IL-11Rα cDNA was cloned by screening a human bone marrow cDNA library with two distinct probes, one specific for the human and one specific for the murine DNA sequences. The human probe, obtained by PCR of TF1 cDNA, specifically hybridized to a family of cDNA clones, all encoding the N-terminal 298 amino acid residues of the IL-11 receptor (depicted as *clone 5'-1* in Fig. 1*A*). Using the murine PCR fragment as probe, two distinct families of cDNA clones were detected. One consisted of clones containing a cDNA insert of about 750 bp encoding part of the cytokine-binding domain including the conserved WSTWS motif, the transmembrane domain, the cytoplasmic domain, and the 3'-nontranslated cDNA (*clone 8e*, Fig. 1*A*). The second family was identical to the first, except that it contained a non-spliced intron at position 1169, the presence of which results in the premature termination of translation, producing a protein that lacks the cytoplasmic domain (*clone rec 3/4*, Fig. 1*B*). Ligation of the cDNA inserts from clone 5'-1 with either clone 8e or clone rec3/4 resulted in the generation of clones encoding the entire human IL-11 receptor protein of 422 amino acids (*clone 5'-8e*, Fig. 1*A*) and a truncated protein of 390 amino acids (*clone 5'rec*, Fig. 1*B*), respectively. The same isoforms of the human IL-11 receptor with identical amino acid sequences have also recently been isolated from human placental and human skeletal muscle cDNA libraries by Cherel *et al.* (28). The intron sequence in clone rec3/4, however, contains a 52-bp insertion at position 1259 in contrast to the published cDNA sequence by Cherel *et al.* (28). The correspondence of our intron sequence to the published genomic DNA sequence (GenBank, U32323) indicates that the sequence is authentic.

The Cytoplasmic Domain of the Human IL-11 Receptor Is Not Necessary for Signal Transduction in Hep3B Cells—In order to assess the functional expression of IL-11Rα, we chose Hep3B hepatoma cells because they are naturally responsive to IL-6 but do not respond to IL-11 due to the absence of endogenous IL-11 receptor α. It has been shown that APRF (acute phase response factor/STAT3) (17) is rapidly activated by IL-6, LIF, OSM, CNTF, and IL-11 (18, 42) and that it binds to IL-6 response elements (IL-6RE) of various plasma protein and immediate-early genes (43, 44). Binding of APRF to these promoters results in their transcriptional activation. We used a construct that carries four tandem copies of the 18-bp IL-6RE core region of the rat α_2-macroglobulin gene in front of its own minimal promoter ($4xT\alpha_2M$) (39), linked to the firefly luciferase reporter gene (40). The cDNA inserts of clones 5'-8e and 5'-rec were subcloned into the mammalian expression vector pHD (pHD-8e and pHD-rec, respectively) and cotransfected together with $4xT\alpha_2M$. After 16 h, the cells were treated with IL-6 or IL-11, and luciferase expression was measured. As shown in Fig. 2, Hep3B cells transfected with the empty vector pHD showed a 4-fold luciferase induction only upon addition of IL-6 but not of IL-11. Transfection of pHD-8e or of pHD-rec (Fig. 2) resulted in the induction of luciferase upon addition of IL-11 that was comparable with that of IL-6. This result indicates that the cytoplasmic domain of the IL-11Rα is not necessary for activation of the JAK-STAT signaling pathway.

Soluble IL-11Rα Is Capable of Inducing Signal Transduction in Hep3B Cells—Two soluble forms of IL-11Rα lacking the transmembrane and the cytoplasmic domain were generated. In the first, the coding region from amino acids 1 to 364 was extended by the addition of a c-MYC-derived epitope to the COOH terminus (35), recognizable by a specific antibody. A second soluble form of the IL-11 receptor was generated with a FLAG epitope directly downstream of the predicted cytokine-binding domain, resulting in the production of a shorter protein terminating at amino acid position 323. Proteins were expressed using the baculovirus system (see "Experimental Procedures" for details), and their production was assessed by Western blot analysis of tissue culture supernatant of cells infected with recombinant baculovirus (Fig. 3). In both cases, the apparent molecular mass was higher than the estimated molecular mass, suggesting that IL-11Rα is subject to post-translational modifications. The amount of protein secreted into the supernatant was comparable with sgp130-myc and sgp130-flag produced with the same method (see Fig. 3, *lanes 2* and *4*).

In order to investigate whether sIL11Rα was biologically active, Hep3B cells were treated for 10 min with either IL-6 or various combinations of IL-11 and sIL11R-myc. After prepara-

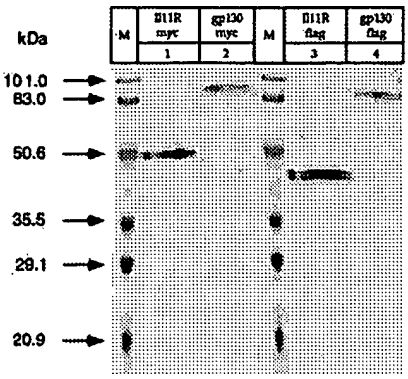

FIG. 3. **Production of sIL11R-myc and sIL11R-flag in insect cells.** Shown is a Western blot analysis of 50 μl of supernatant of High Five cells infected with recombinant baculovirus expressing sIL11R-myc (*lane 1*), sgp130-myc (*lane 2*), sIL11R-flag (*lane 3*), and sgp130-flag (*lane 4*). *M*, molecular mass marker. After electrophoresis through a 12% SDS-PAGE, the proteins were electroblotted to cellulose nitrate membranes and detected by incubation with anti-myc 9E10 (*lanes 1* and *2*) or anti-flag (*lanes 3* and *4*) monoclonal antibodies. An anti-mouse IgG conjugated to alkaline phosphatase was employed for immunostaining. The *double band* visible in *lane 3* might be due to different forms of glycosylation.

tion of whole cell extracts, the DNA-binding activity of APRF was examined by gel retardation assays using a ^{32}P-labeled synthetic oligonucleotide containing the APRF binding site (41) as probe. Fig. 4 shows that neither IL-11 nor sIL11R-myc alone caused APRF activation in these cells. However, a combination of IL-11 together with sIL11Rα induced APRF binding, similar to that induced by IL-6. It can thus be concluded that soluble IL-11Rα is capable of assembling functional receptor complexes and of triggering the JAK-STAT signaling cascade.

In Vitro Binding of IL-11 to the Soluble IL-11Rα Is Specific and Potentiated by sgp130—sIL11Rα forms were used for *in vitro* receptor assembly assays (31). sIL11R-myc was immobilized on protein A-Sepharose beads coated with specific anti-myc monoclonal antibodies and incubated with various combinations of labeled IL-11, labeled IL-6 and labeled or unlabeled sgp130-flag (Fig. 5). Labeled IL-11 was able to bind sIL11R-myc, and binding was increased in the presence of sgp130 (compare Fig. 5, *lanes 1* and *2*). Binding is specific, as labeled IL-6 could not be immunoprecipitated by sIL11R-myc (Fig. 5, *lane 3*). gp130 is part of the IL-11 receptor complex, as labeled gp130 was immunoprecipitated by sIL11R-myc in the presence of IL-11 (Fig. 5, *lane 4*).

The IL-6 receptor system was used as a control. sIL6R-myc specifically immunoprecipitated IL-6 and, as also shown for IL-11, binding of IL-6 increased in the presence of sgp130-flag (Fig. 5, *lane 5* and *6*). sIL6R-myc, however, did not immunoprecipitate IL-11, demonstrating the specificity of binding of IL-11 to its receptor (Fig. 5, *lane 7*).

Higher Order In Vitro Assembly of the IL-11 Receptor Complex—We and others (30, 31) have recently shown that for both IL-6 and CNTF (32) the complex assembled *in vitro* is a hexamer formed by two molecules of the cytokine, two α-chains and two β-chains. We therefore decided to analyze whether the IL-11 receptor complex has a similar composition. In order to assess receptor-α and cytokine dimerization, immunoprecipitation experiments were performed in which either sIL11R-myc or IL-11-flag was first bound to protein A-Sepharose beads by specific monoclonal antibodies and then incubated with a combination of other components as indicated (Fig. 6). The co-immunoprecipitation of ^{35}S-labeled sIL11R-flag together with sIL11R-myc was strictly dependent on both soluble gp130 and IL-11 (Fig. 6, *lane 1*). This event is very reminiscent of IL-6Rα

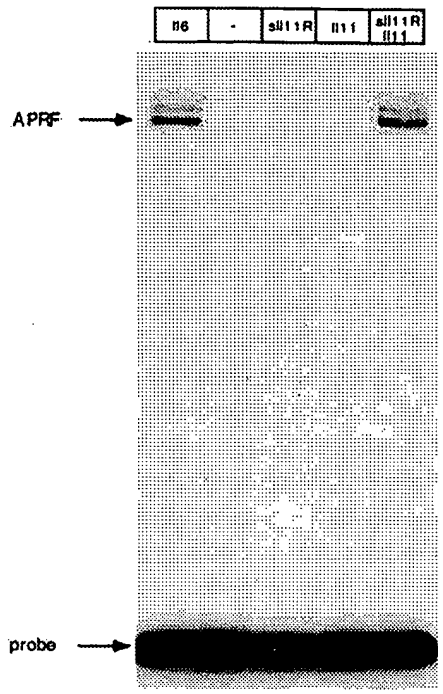

FIG. 4. **The soluble form of the IL-11 receptor α is able to activate APRF in Hep3B cells in the presence of IL-11.** Hep3B cells were treated either with medium alone (−) or with IL-6 (100 ng/ml), IL-11 (100 ng/ml IL11-flag, see "Experimental Procedures"), sIL11R-myc (250 μl of supernatant/ml), or a combination of IL11-flag and sIL11R-myc for 10 min, and proteins (10 μg) were analyzed for APRF activity in a gel retardation assay. After incubation with a radiolabeled synthetic oligonucleotide containing the APRF binding site of the rat α_2-macroglobulin promoter, DNA-protein complexes were separated by electrophoresis through a native 5% polyacrylamide gel and visualized by autoradiography.

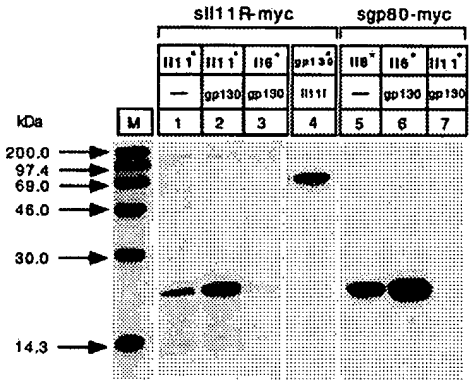

FIG. 5. **IL-11 specifically binds to the soluble IL-11 receptor *in vitro*.** Cold sIL11R-myc (*lanes 1–4*) or sIL6R-myc (*lanes 5–7*) were attached to protein A-Sepharose beads via anti-myc 9E10 monoclonal antibodies. Immunoprecipitation experiments were carried out in the presence of the molecules indicated on *top* of each lane in the following amounts: 30 μl of ^{35}S-labeled IL-11 or IL-6, 50 μl ^{35}S-labeled gp130-flag, 50 μl of cold IL11-flag, 300 μl of cold gp130-flag in a final volume of 350 μl. *sgp80-myc*, sIL6R-myc; *gp130*, sgp130-flag; *IL11f*, IL11-flag; *M*, molecular mass marker. *Asterisks* indicate ^{35}S-labeled protein. Shown is the autoradiograph of the dried gel. *Lanes 1–3* represent a three times longer exposure with respect to *lanes 4–7*.

dimerization (31).

A similar result was obtained for the cytokine: ^{35}S-labeled IL-11 was co-immunoprecipitated by immobilized IL11-flag only in the presence of sgp130 and sIL11Rα (Fig. 6, compare *lane 4* with *lanes 5* and *6*).

Further experiments were performed to assess dimerization

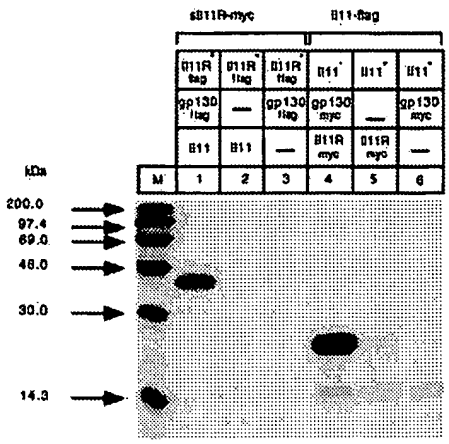

FIG. 6. **IL-11 receptor α and IL-11 dimerize *in vitro*.** Cold sIL11R-myc (*lanes 1–3*) and IL11-flag (*lanes 4–6*) were immobilized on protein A-Sepharose via anti-myc 9E10 and anti-FLAG M2 monoclonal antibodies, respectively. Immunoprecipitation was carried out in the presence of the molecules indicated on *top* of each lane with the following amounts: 80 μl of ^{35}S-labeled sIL11R-flag, 300 μl of cold sgp130-flag, 50 μl of IL11-flag, 30 μl of ^{35}S-labeled IL-11, 300 μl of cold sgp130-myc, and 300 μl of cold sIL11R-myc. *IL11*, IL11-flag. *M*, molecular mass marker. *Asterisks* indicate ^{35}S-labeled protein. Shown is the autoradiograph of the dried gel.

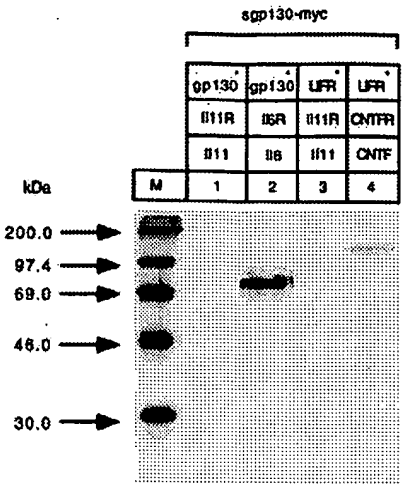

FIG. 7. **Neither a homodimer of gp130 nor a heterodimer of gp130 and LIFR are detected *in vitro*.** Cold sgp130-myc was immobilized on protein A-Sepharose via anti-myc 9E10 monoclonal antibodies and incubated with the molecules indicated on the *top* of each lane in the following amounts: 300 μl of sIL11R-flag, 50 μl of IL11-flag, 50 μl of ^{35}S-labeled sgp130-flag, 300 μl of sIL6R-flag, 10 μg of IL-6, 50 μl of ^{35}S-labeled LIFR, 300 μl of CNTFR, and 1 μg of CNTF. *IL11R*, sIL11R-flag; *IL11*, IL11-flag; *gp130*, sgp130-flag; *IL6R*, sIL6R-flag; *LIFR*, sLIFR-flag; *CNTFR*, sCNTFR-flag; M, molecular mass marker. *Asterisks* indicate ^{35}S-labeled protein. Shown is the autoradiograph of the dried gel.

of the signaling chains, either gp130 homodimerization or gp130-LIFR heterodimerization. sgp130-myc was immobilized onto protein A-Sepharose beads coated with anti-myc monoclonal antibodies and incubated with IL-6 and sIL-6Rα. This led to the immunoprecipitation of ^{35}S-labeled sgp130-FLAG (Fig. 7, *lane 2*), an indication of *in vitro* gp130 homodimerization (31). In contrast, no evidence for gp130 homodimerization was obtained using a combination of IL-11 and sIL11R (Fig. 7, *lane 1*); not even increasing the amounts of soluble IL-11 receptor or IL-11 cytokine in the immunoprecipitation experiment or using gp130-flag immobilized on protein A-Sepharose resulted in the binding of a second soluble gp130 chain (data not shown). Using the same assay conditions, heterodimerization of gp130 with LIFR was tested. As expected, labeled LIFR did not heterodimerize with gp130-myc in the IL-11 receptor complex *in vitro*, in contrast to the CNTF receptor complex, where LIFR was detectable in the presence of CNTF receptor and CNTF (compare Fig. 7, *lanes 3* and *4*).

DISCUSSION

The biological and structural properties (45) of IL-11 together with the data from the cloning and expression of the murine IL-11 receptor (26) have identified this cytokine as being a member of the IL-6 cytokine family. In this work we expressed the human IL-11 receptor α-chain and studied the composition of the IL-11 receptor complex. Two different cDNA isoforms were isolated from a human bone marrow cDNA library. The first one encoded the entire IL-11 receptor, and the second contained a non-spliced intron at the C-terminal end of the transmembrane domain and encoded a truncated protein containing the entire N-terminal part of the protein, including the transmembrane domain, but lacking the cytoplasmic domain.

Alternative splicing is a phenomenon often observed in members of the cytokine/hematopoietin receptor family (46, 47), which results in receptor heterogeneity through expressing different cytoplasmic or extracellular domains or by generating soluble variants. In the latter case, however, all alternatively spliced proteins terminate before the transmembrane domain. In the case of IL-11, the shorter IL-11 receptor isoform identified in the present work encodes almost the entire transmembrane domain except for the last amino acid, and therefore it is unlikely that the corresponding protein represents a soluble form of the IL-11 receptor α. Indeed, a similarly spliced isoform has also been identified for the murine IL-4 receptor (48) and has been shown to be integrated into the membrane and expressed on the cell surface.

Transfection experiments in Hep3B cells showed that the IL-11 receptor was able to confer IL-11 responsiveness to this naturally non-responsive cell line. As expected from analogy with the IL-6 system, the shorter isoform lacking the intracytoplasmic domain was equally effective. Hence IL-11Rα, like IL-6Rα, acts as a docking molecule that traps the cytokine on the cell surface but does not contain the information required for signal transduction in its intracytoplasmic domain. This conclusion is further reinforced by the evidence that a soluble form of IL-11Rα is biologically active in conferring IL-11 responsiveness to the same cells. It is known that sIL-6Rα, shed from the surface of expressing cells, circulates in the blood in detectable amounts. This soluble form of IL-6Rα is believed to play an important role in physiological and pathological conditions by acting as a cytokine chaperone and as a potentiator of its activity (47). On the basis of our findings it will be important to determine whether sIL-11Rα is also released into biological fluids, which could explain some of the biological properties of the cytokine *in vivo*.

The availability of sIL-11Rα produced at high levels in the baculovirus expression system has made it possible for the first time to perform biochemical studies of IL-11 receptor assembly *in vitro*. As expected, sIL-11Rα specifically and directly interacts with IL-11, and this binding is potentiated (probably stabilized) by gp130. When higher order receptor assembly was explored and compared with IL-6 and CNTF, important analogies and differences were observed. The IL-11·IL-11Rα subcomplex binds to gp130 as expected from previous studies with cell lines (26), and the IL-11·IL-11R subcomplex was shown to undergo dimerization in the presence of gp130. However, this latter molecule did not homodimerize, as is the case for IL-6. It is unlikely that β-chain dimerization has not been detected due

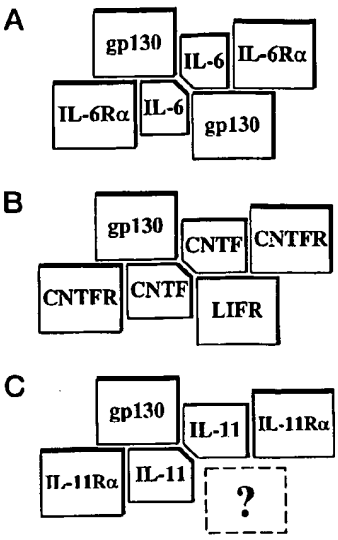

FIG. 8. **Models of the hexameric cytokine receptor complexes.** *A*, model of the IL-6 hexameric receptor complex. *B*, proposed model of the CNTF hexameric receptor complex. *C*, proposed model of a hexameric IL-11 receptor complex in which is indicated the second, as yet unknown, signal-transducing β-component.

to technical reasons, as relevant controls were run in parallel and sIL-11Rα preparations were shown to be biologically active. The fidelity of the *in vitro* binding assays is further proven by the lack of binding to LIFR, previously shown to be not required for IL-11 signaling (26, see also transfection experiments into Hep3B cells presented in this study, which are known to lack expression of LIFR). Based on our results, therefore, IL-11, IL-11Rα, and gp130 assemble a pentameric complex *in vitro* composed of two IL-11, two IL-11Rα, and one gp130 molecule. Since β-chain dimerization is essential for cytokine signaling, it is highly likely that another still unidentified β-chain of the gp130 type contributes to the formation of a hexameric high affinity IL-11 receptor complex (Fig. 8). The identification of this additional chain and its expression pattern will help us to better understand the biology of IL-11 and to distinguish which functions of this cytokine are unique or common to the other members of this family.

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